Automatic Feeder for Newborn Rat Use Within 12 Hours of Birth

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Abstract | An automatic feeder was developed for rat pups that were nursed by mother rats during the first three days of life. Now, an automatic feeder has been developed for feeding newborn rat pups within 12 h of birth. In this apparatus, the nipples projected vertically downward. When pups were placed in the apparatus, all assumed the supine position. A few pups established nipple attachment by themselves without training. The remaining pups were trained with nursing bottles until they established nipple attachment. By 2 days after starting training, all pups found nipples and suckled milk by themselves.

The general method for artificially rearing rat pups was originally developed by Messer et al. (1), and was later modified by Hall (2). This procedure of rearing rat pups by feeding them exclusively via chronic intragastric cannula has been used for many studies of nutrition and behavior (3-8). However, this method has some disadvantages, including deprivation of maternal and sibling social interactions and behavior, such as suckling and swallowing milk.

An automatic feeder was developed for rat pups that were nursed by mother rats during the first three days of life (9), indicating that pups could be reared automatically after the training procedure. Having repeated the procedure 3 to 4 times, pups began to seek the nipples frequently, and after 3 to 5 additional times, all pups found the nipples and suckled milk by themselves.

Using this apparatus, an attempt was made to artificially nurse newborn rats within 12 h of birth; however they could not find the nipples and suckle milk by themselves, even with training. Blass et al. (10) reported cues such as chemical and tactile stimuli, with which information about nipple location is provided to pups. They mentioned that maternal temperature, thermal gradient, and the contrast with the mother rat's fur were unlikely cues for eliciting suckling. Their data indicated that the first nipple attachment is elicited by maternal saliva and amniotic fluid coating the nipples when the dam licked the pups and performed self grooming during parturition. Furthermore, according to their data, the necessary and sufficient condition for nipple attachment is that the nipple be coated with saliva of pups after the first suckling experience.

On the basis of these findings, an attempt was made to develop an automatic feeder for nursing newborn rats within 12 h of birth.

Materials and Methods

Automatic feeder: Basically, the apparatus for newborn rats was similar to that for 8-day-old pups, except for the direction and structure of the nipples. In the apparatus for 8-day-old pups, the nipples projected slightly downward (9); on the other hand, in the apparatus for newborn rats, the nipples projected vertically downward.

The apparatus consisted of a nursing bottle-milk tank unit, container, nylon fur, upper case (14.8 cm long, 7.0 cm high, and 9.0 cm wide, made of polyvinyl chloride boards, 2 cm thick), sheet of carpet (14 cm long, 0.8 cm high, and 8.0 cm wide), polyvinyl chloride boards (0.25 mm thick), bottom case (14.4 cm long, 8.0 cm high, and 8.5 cm wide, made of polyvinyl chloride boards, 2 cm thick), and water bath (Figures 1 and 2).

The nursing bottle was a glass tube, 6 cm long and 1.4 cm in diameter, closed at one end with a silicone stopper in which a 2-mm-diameter hole was bored. The nipple consisted of inner and outer nipples. The outer nipple was fixed to a silicone stopper, and the inner nipple was introduced into the outer nipple and glued in place with silicone glue. Nipples were made of a silicone rubber (FSLS, Fuji Systems Corporation, Tokyo, Japan) on aluminum forms (Figure 3). The wide part of form A was 1.6 cm in diameter and 4 cm long; the narrow part was 1.1 mm in diameter and 4.6 cm long. The wide part of form B was 1.6 cm in diameter and 4 cm long; the narrow part was 1.5 mm in diameter and 5.8 mm long.

The forms were dipped in silicone rubber and were solidified at about 130°C for 15 min in an oven (Convection oven, MOV-102F, Sanyo Electronic, Co. Ltd., Osaka, Japan). Dipping and solidification was repeated (4 to 7 times), and the thickness of the nipple was regulated by the number of dippings. The outer nipple was 5 mm long and 1.5 mm in inner diameter, and the inner nipple was 3 mm long and 1.0 mm in inner diameter. The thickness of both nipples was 0.3 to 0.4 mm. These nipples were used for pups up to 5 days old. From 6 to 10 days of age, the length of outer nipple was increased to 6.5 mm and the thickness of the outer nipple was 0.6 to 0.7 mm. The tip of the outer nipple was cut crossways. Four slits, 1.5 mm long, were made on the side of the inner nipple. Milk flow rate was controlled by the length (0.5 to 0.9 cm) and inner diameter of the needle (24 to 26 gauge) inserted into the inner nipple. The nursing bottle was divided by a silicone partition that was positioned 1.5 cm from the end of the glass tube on the nipple side.

Two silicone tubes (Fuji Systems Corporation), 1 mm in inner diameter and 2 mm in outer diameter, were inserted into the nipple side of the glass tube through the partition, one for milk inflow and the other for milk outflow. The tube for milk outflow was designed to keep the milk level below the partition by recovering milk into a milk tank (200-ml flask), using a peristaltic pump, maintaining the flow rate of milk from the nipple constant. An opening for an air inlet and outlet was provided in the partition. The nursing bottle-milk tank unit (Figure 2A), with the exception of the peristaltic pump, was autoclaved at 121°C for 20 min and exchanged with a clean one twice a day.

A container, 14.5 cm long, 3.5 cm high, and 8.5 cm wide, was made of polyvinyl chloride boards, 3 mm thick (Eslon plate®, Sekisui Chemical Co. Ltd Osaka, Japan) (Figure 2B). Six holes, 1.6 cm in diameter, were made in the upper and bottom boards. Six copper pipes, 1.6 cm in outer diameter and 4.5 cm long, were passed through the holes until one rim was level with the bottom surface. The opposite open end projected 1 cm from the upper board. Nursing bottles were inserted into the pipes until the nipples protruded in place and were held with silicone rings at the upper rims of the pipes. The nipples were arranged in two rows (Figure 4). The distance between the nipples was 2 cm. Hot water, 35 to 40°C, was circulated through the container from the water bath (Auto-Temper® GW-901, Thermal Kagaku Sangyo, Tokyo, Japan) (Figure 2H). Temperature around the nipples was maintained at 33 ± 2°C by use of a thermostat. The sensor was located near the nipples, which were surrounded by nylon fur (Magic Tape® Kuraray Co. Ltd., Tokyo, Japan) (Figures 2C and 4). It was exchanged when soiled for the first three days and twice a day thereafter.

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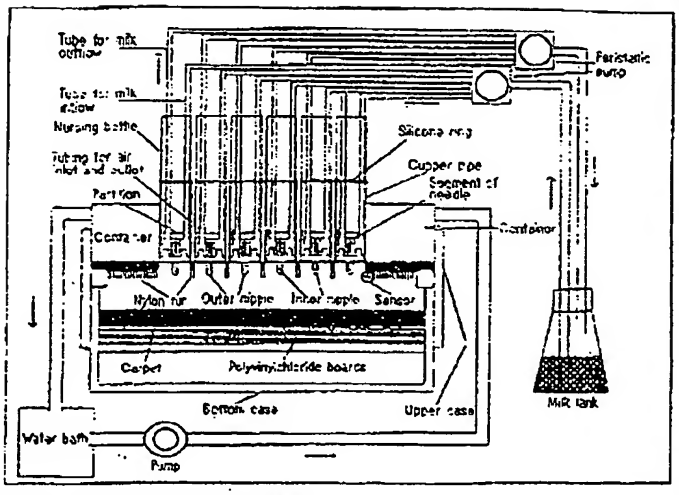

FIG. 1. Design of automatic feeder.

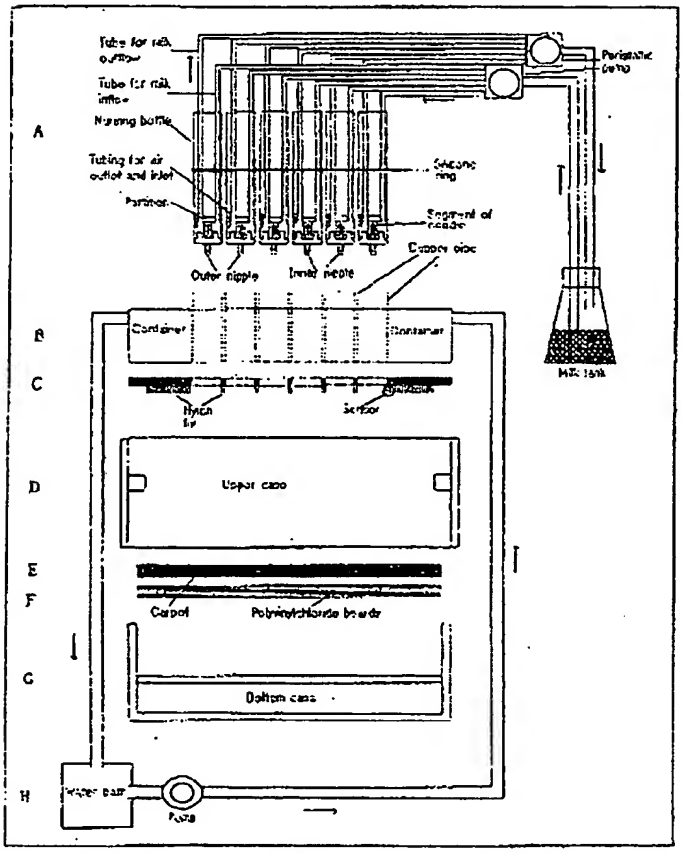

FIG. 2. Drawing of an internal view of the automatic feeder to show isolated components. A = nursing bottle-milk tank unit; B = container; C = nylon fur; D = upper case; E = sheet of carpet(bedding); F = polyvinyl chloride boards (0.25 mm thick); G = bottom case; H = water bath.

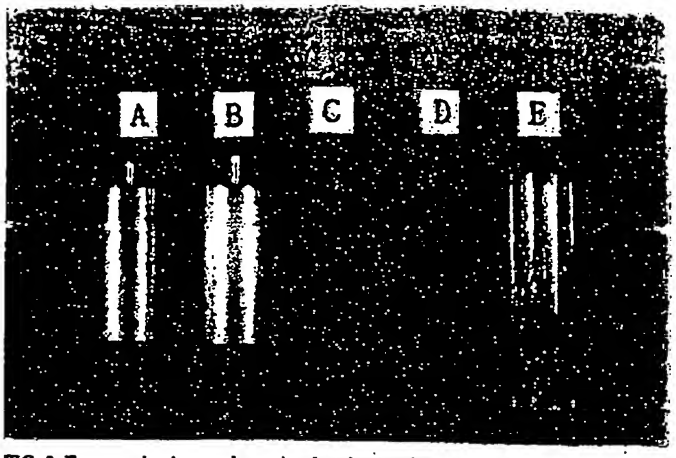

FIG. 3. Forms, nipples, and nursing bottle. A = form for newborn to 5-day-old pups; B = form for 6- to 10-day-old pups; C = nipple fixed to a silicone stopper for newborn to 5-day-old pups; D = nipple fixed to a silicone stopper for 6- to 10-day-old pups; E = nursing bottle.

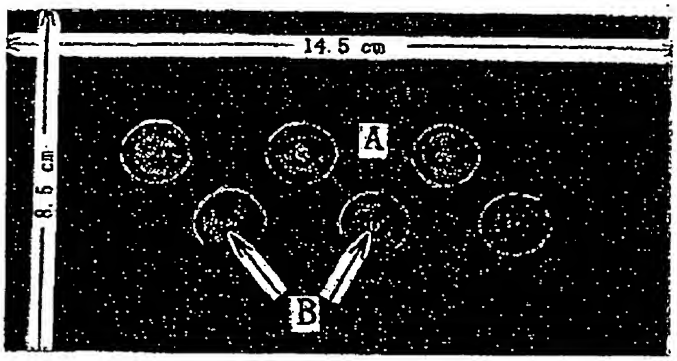

FIG. 4. Nylon fur around the nipples. A = nylon fur; B = nipples.

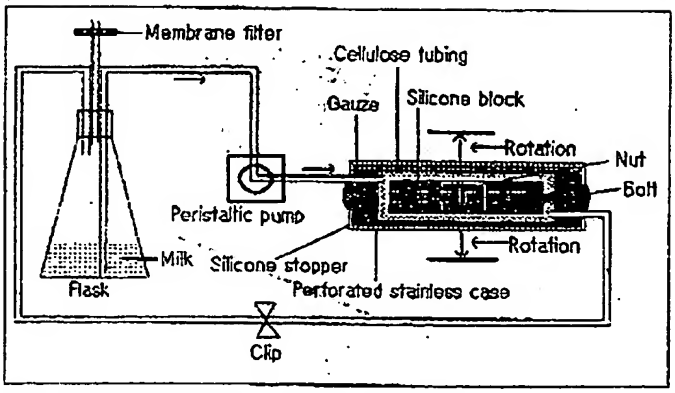

FIG. 5. Design of apparatus for concentration of milk.

A sheet of carpet made of acrylic fiber (easily available at retail stores), was used as bedding and exchanged twice daily (Figure 2E). It was positioned in the bottom case (Figure 2G). The distance from the bedding to the nipple was approximately the same as that from the abdomen to the back of the pup. It was adjusted to accommodate growth by increasing or decreasing the number of polyvinyl chloride boards (0.25 mm thick) that were located beneath the bedding (Figure 2F).

Milk: For an automatic feeder to function well, satisfactory substitute milk is necessary. According to a method modified from Smart et al. (11), a milk substitute was produced by filtration, using cellulose tubing (Figure 5). Cellulose tubing (28 cm) was washed for 4 h in running tap water. Both ends of the cellulose tubing were plugged with silicone stoppers and tied tightly. A silicone block, 15 cm long and 6 cm in diameter, was placed in the 300-ml cellulose tubing. The

tubing was covered with cotton cloth and a perforated stainless case to prevent rupture. Milk flowed from a flask (1,000 ml) to the cellulose tubing by use of a peristaltic pup. The stainless case containing the cellulose tubing was rotated from side to side slowly. Cows' milk pasteurized at 140°C for 3 sec (Meiji Milk Products, Co. Ltd., Tokyo, Japan) was purchased commercially and the volume was decreased from 1,000 ml to 300 ml for 2 days. An amino acid mixture containing vitamin C was sterilized by passing it through a filter (Millipore®, 0.3 μm, Nihon Millipore Kogyo, Yonezawa, Japan). It contains the following amounts per 100 ml of water; l-methionine, 1 g; l-arginine, 1 g; l-tryptophane, 1 g; glycine, 1 g; and sodium l-ascorbate, 1 g. Ten milliliters of this mixture was added to 90 ml of the concentrated milk immediately before use. The gross composition of the milk substitute is shown in Table 1.

Urination: The mother rat frequently licks the peritoneal region of the pup to stimulate urination and defecation. Therefore, routine procedure included stroking of the urethral papilla and the anal region, using a piece of cotton wool. This procedure was repeated at 3-h intervals from 9 AM to 9 PM, except at night.

Training procedure: Pups were grasped by the neck and held between the thumb and the forefinger, then were nursed with the nursing bottle at 3-h intervals from 9 AM to 9 PM, except during the night until they suckled milk by themselves.

Animals: The Sprague-Dawley rats used were the same strain as that used in the previous report (9). They were maintained by sister-brother matings at the Animal Center for Medical Research, Okayama University Medical School since 1975. They were fed commercially prepared diet (NMF®, Oriental Yeast Co. Ltd., Osaka, Japan) and water ad libitum. Pups removed within 12 h after birth were reared by use of the automatic feeder until 10 days of age. Five experiments using five animals each were performed. Mother-nursed pups served as control. Animal care procedures were performed according to the guidelines of the Okayama University Medical school.

Results

When newborn pups were placed in the apparatus, all assumed the supine position and began "rooting behavior," as described by Blass et al. (10). Namely, the pups moved quickly across the skin and fur of the automatic feeder, using swimming-like motions and scanned their environment by moving their head from side to side. A few pups established the first nipple attachment by themselves without training. The remaining pups that could not accomplish it, were nursed by nursing bottle until they established the nipple attachment by themselves. By 2 days after starting the training, all pups found nipples and suckled milk by themselves (Figure 6). Growth curves of the automatic feeder-reared and the mother-nursed pups are shown in Figure 7. The former gained less weight than did the mother-nursed pups. Three of the 25 automatic feeder-reared pups died. In the previous study, automatic feeder-reared pups that had been nursed by mother rats during the first three days gained less weight than did mother-nursed pups until 11 days. After that, almost all pups had sufficient weight gain, compared with mother-nursed pups. At the time of weaning (20 days), the automatic feeder-reared pups were heavier than the mother-nursed pups. When pups reared by use of this new apparatus until 10 days of age were placed in the apparatus used in the previous study, they found the nipple and suckled milk by themselves without training or after a few training sessions.

Table 1. Gross composition of substitute milk

Protein (%)	9.54
Fat (%)	9.90
Lactose (%)	3.79
Ash (%)	1.09
Water (%)	75.68

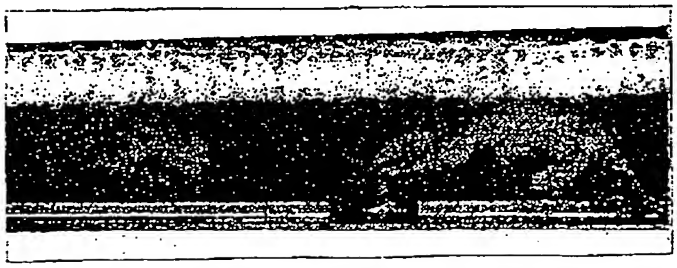

FIG. 6. Rat pups suckling milk automatically. A part of the skin and fur is cut off to show pups attaching to nipples.

Discussion

Using the previously described feeder (9), 3-day-old pups suckled milk in standing position. However, newborn pups within 12 h of birth could not find the nipples and suckle milk by themselves, even after training. It was observed that newborn rats suckle milk in the supine position with the dam crouching over them. From this observation, the apparatus was improved by having the nipples project perpendicularly downward. When newborn pups were placed in this apparatus, they assumed the supine position and exhibited "rooting behavior." A few pups found nipples and suckled milk by themselves without training. From this result, it was suggested that the "rooting behavior" was hereditary. In the previous study, 3-day-old rats did not exhibit "rooting behavior" or suckle milk without training. In the new apparatus, newborn and 3-day-old rats began "rooting behavior" without training. From these results, the supine position was considered essential for newborn pups to be able to suckle milk by themselves.

Few newborn rats gained weight by consuming the milk substitute used for 3-day-old pups (9). This probably was due to maldigestion of the milk. Generally, enzyme treatment was used to improve the digestibility of milk. It has been documented that curd consistency is an index of the time required for gastric digestion (12). In the preliminary study, enzyme treatment was performed using various concentrations of trypsin, and intense sonic vibration was applied to reduce curd tension (data not shown). The result was inconsistent. Almost all of one group of pups gained weight. None of the pups in the other group gained weight. Possible causes of these results include: the viscosity of milk substitute varied with each lot, which resulted in difficulty in controlling the flow rate from the nipple; and curd tension of milk in the stomach varied with each lot, result-

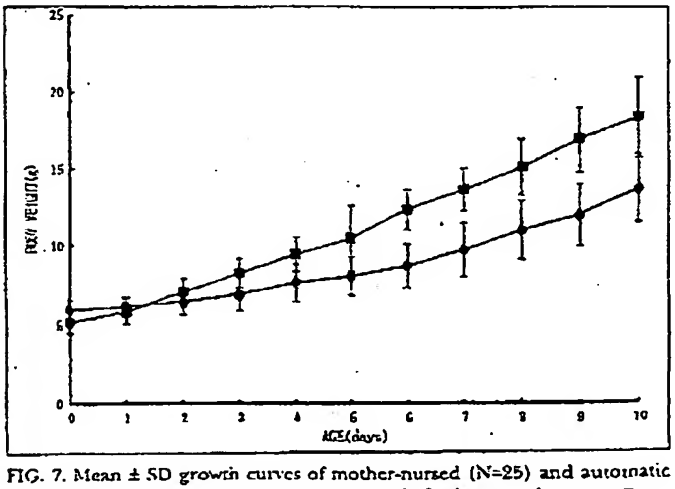

FIG. 7. Mean ± SD growth curves of mother-nursed (N=25) and automatic feeder-reared pups (N=25). – ● – = Automatic feeder-reared pups; – ■ – = mother-nursed pups.

ing in maldigestion of milk. Smart et al. (11) developed a milk substitute for rat pups and reported that pups consuming it via gastric cannula had satisfactory weight gain, compared with that in mother-nursed pups. This milk was produced by ultrafiltration of pasteurized cows' milk. Success using this procedure was explained by the casein micelles and fat globules of the original milk remaining intact, so that formation of a normal coagulum during digestion was likely to be facilitated. In this study, according to a method modified from Smart et al., a new milk substitute was produced and used successfully to nurse newborn pups within 12 h of birth.

Blass et al. (10) reported that the cue necessary for suckling was the nipple coated with saliva of pups after the first suckling experience. Furthermore, their data indicated that the first attachment was elicited by maternal saliva and amniotic fluid coating the nipples when the mother licked the pups and performed self-grooming during parturition. In the study reported here, some pups had already accomplished the first attachment to the mother's nipple and the others had not. When pups were placed in the apparatus, a few accomplished nipple attachment by themselves, even if the nipple was not coated with saliva and amniotic fluid. Pups that could not establish nipple attachment by themselves, were nursed by nursing bottle at 3-h intervals from 9 AM to 9 PM, except during the night. All pups found the nipples and suckled milk by themselves within about two days. After the first suckling experience using this apparatus, the skin, fur, and nipples were exchanged twice a day with clean ones that were not coated with saliva of pups. From these findings, after the first nipple attachment, saliva of pups is not necessarily the essential cue in this apparatus. Furthermore, the finding of Blass et al. (10) that the first nipple attachment is elicited by both maternal saliva and amniotic fluid raises the question of whether the pups can be attracted by the skin of each other and themselves, as they have been coated with maternal saliva and amniotic fluid when the mother licks her pups during parturition. This apparatus may be potentially useful in studies of nutrition, behavior, immunology, psychology, and toxicology.

Acknowledgement

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References

1. Messer, M., E. B. Thoman, A. G. Terrasa, *et al.* 1969. Artificial feeding of infant rats by continuous gastric infusion. J. Nutr. 98:404–410.
2. Hall, W. G. 1975. Weaning and growth of artificially reared rats. Science. 190:1313–1315.
3. Smart, J. L., D. N. Stephene, J. Tonkiss, *et al.* 1984. Growth and development of rats artificially reared on different milk-substitutes. Br. J. Nutr. 52:227–237.
4. Tonkiss, J., J. L. Smart, and R. F. Massey. 1987. Growth and development of rats artificially reared on rats' milk or rats' milk/milk-substitute combinations. Br. J. Nutr. 57:3–11.
5. Smart, J. L., R. F. Massey, S. C. Nash, *et al.* 1987. Effects of early-life undernutrition in artificially reared rats: subsequent body and organ growth. Br. J. Nutr. 58:245–255.
6. Moats-Staats, B. M., J. L. Brady, Jr., L. E. Underwood, *et al.* 1989. Dietary protein restriction in artificially reared neonatal rats causes a reduction of insulin-like growth factor-I gene expression. Endocrinology 125(5):2368–2374.
7. Patel, M. S., and B. K. Hiremagalur. 1992. Artificial-rearing technique: Its usefulness in nutrition research. J. Nutr. 122:412–419.
8. Hiremagalur, B. K., G. L. Johanning, S. C. Kalhan, *et al.* 1992. Alterations in hepatic lipogenic capacity in rat pups artificially reared on a milk-substitute formula high in carbohydrate or medium-chain triacylglycerides. J. Nutr. Biochem. 3:474–479.
9. Hoshiba, J. 1986. An automatic feeder for infant rats. Lab. Anim. Sci. 36 (No. 6):682–685.
10. Blass, E. M., and M. H. Teicher. 1980. Sucking. Science 210:15–22.
11. Smart, J. L., R.F. Massey, and A.C. McMahon. 1992. Ultrafiltered cow's milk as a milk substitute for rat pups. Proc. Nutr. Soc. 51:40A
12. Chambers, L. A. 1936. Soft curd character induced in milk by intense sonic vibration. J. Dairy Sci. 19:2947.